

In conclusion, the author stated it was impossible in a short abstract to go into the details of the subjects here discussed, and for further information the reader must be referred to the paper itself.

II. "Physiological Action of the Active Principle of the Seeds of *Abrus precatorius* (Jequirity)." By SIDNEY MARTIN, M.D. London, British Medical Association Research Scholar, Assistant Physician to the Victoria Park Chest Hospital, and R. NORRIS WOLFENDEN, M.D. (Cantab.). (From the Physiological Laboratory, University College.) Communicated by E. A. SCHÄFER, F.R.S. Received April 11, 1889.

The object of the present investigation was to study the physiological action of the active principle of the jequirity seed. A watery infusion of the seeds, as is well known, produces severe inflammation of the conjunctiva when a few drops are placed in the eye; and when injected under the skin, or as in the "sui" poisoning of cattle in India, it is fatal to animals.

Both the local irritant and the poisonous properties of the seed were formerly ascribed to a specific organism, called the jequirity bacillus, the nature of which was investigated by Sattler, Cornil, and Berlioz. Klein, however, showed that the action could not be due to a bacillus, since the poison was permanently destroyed by momentary boiling of the infusion. Warden and Waddell have effectually disposed of the bacillus theory of the action of jequirity, and in a pamphlet entitled the 'Non-Bacillar Nature of Abrus Poison' (Calcutta, 1884), they demonstrated that the poisonous activity of the seeds was dependent on a proteid body which was called by them *Abrin*. Abrin was considered to be closely allied to egg-albumin and the vegetable albumins. The reactions given by Warden and Waddell are, however, by no means conclusive that abrin belongs to the class of "albumins" as understood by physiological chemists. The fact that it is precipitated from solution by acetic acid shows that it is not an albumin: this is a reaction common to globulin and certain other proteids, such as alkali-albumin. The reactions, moreover, given by these observers as given by abrin are not distinctive of it, but are common to all proteids.

To clear up these discrepancies the proteids of the seed were investigated by one of us (M.), and in a paper published in the 'Proceedings of the Royal Society' (vol. 42, p. 331) two proteids were described, a globulin and an albumose. The globulin was found to be vegetable paraglobulin, being soluble in 15 per cent. sodium chloride solution, and coagulating in 10 per cent. magnesium sulphate

solution between 75° and 80° C. The albumose gave the general reactions of its class, and corresponded to what has been called by one of us (M.) α -phytalbumose.*

The physiological action of the globulin has been investigated by us. It was isolated by the method detailed in the paper already quoted. As the method employed is important, it is again mentioned here. Both globulin and albumose were extracted from the decorticated and crushed seed by a 15 per cent. solution of sodium chloride, and the clear extract saturated with sodium chloride or ammonium sulphate after acidulation with acetic acid. The copious saturation precipitate consists of globulin and albumose: it is dissolved in distilled water, and dialysed in running water until the globulin is in great part thrown down. The precipitate of globulin so obtained is separated by filtration and washed on the filter with previously boiled distilled water until no soluble proteid is present in the washings. The distilled water removes the salt clinging to the globulin and the small amount of albumose present. After this thorough washing the globulin which remains on the filter is a pure proteid. It is removed from the filter and dried over sulphuric acid. If it has been precipitated by ammonium sulphate, the globulin dries in greenish-black scales, owing to the admixture of colouring matter; but if sodium chloride has been used, it is obtained as a whitish-yellow amorphous powder. This was the powder used in our experiments. It consists of pure globulin, almost completely soluble in 15 per cent. sodium chloride solution. A small portion of the proteid became insoluble after dialysing and drying over sulphuric acid.

The solution used for injection was one made with 15 per cent. NaCl solution, which was previously boiled and cooled.

Physiological Action of the Globulin. *The Globulin of the Seeds of the Jequirity has the same Physiological Action as the Watery Extract of the Seeds and as the Proteid body "Abrin" described by Warden and Waddell.*

Local Action.—A watery infusion of the seeds when placed on the conjunctiva causes inflammation of the membrane with purulent discharge. A similar effect is produced by "abrin," and precisely a similar effect by the pure globulin. This severe local action of abrus seeds we therefore ascribe to the globulin of the seeds.

In one experiment, for example, about 0.002 gram of the pure globulin was placed on the inner surface of the left eyelid of a large rabbit. In 17 $\frac{3}{4}$ hours the conjunctiva was reddened and slightly swollen; there was no chemosis, but there was a clear serous discharge from the eye. In twenty-four hours more there was intense

* See "The Nature of Papain, &c.," 'Journ. of Physiology,' vol. 6, p. 344.

purulent ophthalmia with subconjunctival ecchymosis; the cornea was quite clear. The purulent discharge lasted till the death of the animal, about eighty-three hours after the inoculation. The animal was apparently ill for about four hours before death. At the *post-mortem* there was severe subconjunctival hæmorrhage, with œdema round the eyeball.

In another experiment, where a very small quantity of the globulin in a 15 per cent. NaCl solution was placed on the conjunctiva, there was inflammation with purulent discharge from the eye in 16½ hours. The inflammation rapidly increased (although there were no general symptoms of poisoning), and began to subside on the sixth day after inoculation. In this case there was no subconjunctival hæmorrhage, the amount of globulin used being less than in the first experiment quoted.

From both these experiments the local action of the globulin is evident: it produces intense inflammation, œdema, and local ecchymosis, and may produce death if the dose is sufficiently large.

General Action.—The local irritant effect is also seen when a solution of the globulin is injected subcutaneously. The connective tissue becomes œdematous over a large area, there is congestion, and if the dose be a large one there are also punctiform ecchymoses. No suppuration is found, probably because the animal does not live long enough.

As regards the general effect on the body produced by abrus-poison, we have not much to add to the account given by Drs. Warden and Waddell in their pamphlet previously quoted. Abrus-globulin produces the same effect as the substance called by these observers "abrin," and as the watery infusion of the juice.

If a small quantity of globulin, 0·0022 gram (0·01 gram per kilo. of body weight), be injected under the skin of a rat weighing 218 grams, symptoms of poisoning begin to appear in about six hours. The animal then seems a little languid, and in a condition impossible to distinguish from sleepiness. It continues in this state, making no voluntary movement, irresponsive to slight external stimuli, and with half-shut eyes. It lies huddled up in its cage, the breathing becomes more rapid, and bloody motions are passed shortly before death, which occurs in about twenty-four hours after inoculation. If the animal is with young it aborts.

Post-mortem there are signs of œdema and ecchymoses at the seat of injection, and punctiform ecchymoses also beneath the peritoneum and sometimes in the lungs. The intestines are congested, sometimes greatly inflamed; the adenoid patches in the mucous membrane are swollen, and submucous ecchymoses are often seen. The blood sometimes remains fluid for a long time, and is sometimes coagulated.

Fatal Dose.—The minimal fatal dose of the globulin has not been determined by us; the smallest dose we have found kill is 0·0022 gram or a dose of 0·01 gram per kilo. of body weight.

This dose took twenty-four hours to kill; a larger dose kills more rapidly, and we have repeatedly demonstrated the fact that the rapidity and intensity of the action of abrus-globulin is proportional to the quantity used. This is seen in the following table of experiments on pigeons:—

Fatal Dose of Abrus-globulin in Pigeons.

Weight of pigeon in grams.	Dose of globulin in grams.	Dose per kilo. of body weight.	Death occurred in
310	0·0031	0·01	About 18 hours.
397	0·15	0·373	„ 8½ „
216	0·2	0·925	„ 5¾ „

In analysing the general symptoms produced by abrus-globulin (abrus-poison) it is seen that there are no convulsions produced and no definite paralysis, only a general weakening, ending gradually in death.

The fact that the poison produces cedema at the seat of injection, and that death is slow, may point to the idea that the cause of death is secondary to the local lesion produced by the poison, the absorption of septic material occurring. This is not so, however, and for several reasons.

In the first place the local cedema is very slight in amount if the dose be small, and yet death occurs. Suppuration, moreover, has not occurred in any of our experiments. Moreover, if the fatal effect really occurred from the absorption of septic material, we should expect a rise of temperature; the animal would be febrile. This is not so; the temperature instead of rising falls in a most remarkable manner. A few observations were made on cats by Warden and Waddell on this point. The animals used by us were pigeons, and the temperature was taken in the rectum every twenty minutes or half hour after the injection of the globulin. At the same time the number of respirations per minute was counted, so that in the chart (fig. 1) the respiration curve can be compared with the temperature curve.

It will be seen from the chart that the temperature, which was 106·8° F. before inoculation, began to fall in less than half an hour after the poison had been injected; that this fall was gradual until two hours after the inoculation, after which time there is a rapid fall until death. The fall of temperature until death was from 106·8° F. to 83° F.; *i.e.*, 23·8° F. or 12·6° C.

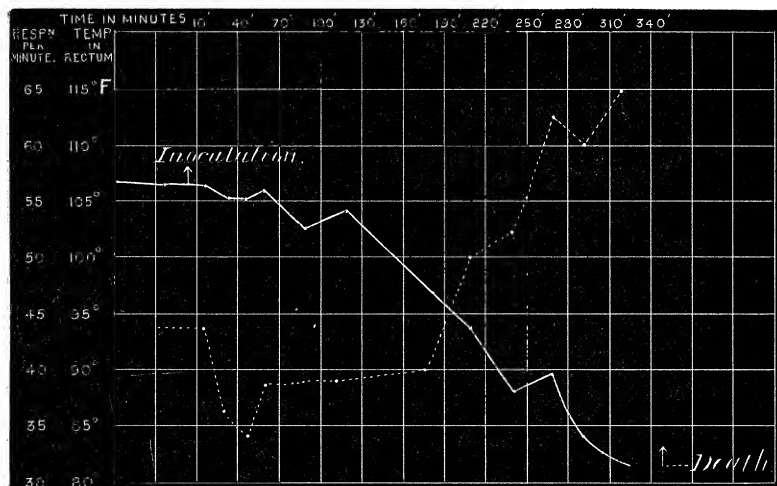


FIG. 1.—Pigeon weighing 216 grams: 0.2 gram abrus-globulin injected over pectoral muscle (0.925 gram per kilo. of body weight). Death in 5 hours 45 mins. Temperature curve, thick line; respiration curve, dotted line.

The respirations are seen from the curve to diminish more rapidly than the temperature falls for fifty-five minutes after inoculation; there is then a stationary period until 115 minutes, after which the number of respirations per minute rapidly rises until death. The curves, therefore, show a fall of temperature with an increase in the number of respirations.

In other experiments, where the temperature has been regularly taken, and the respirations counted, the same phenomena have been observed.

In an experiment where a pigeon weighing 397 grams was inoculated with 0.15 gram of abrus-globulin (0.373 gram per kilo. of body weight), the fall of temperature was at first very rapid; from 107.1° F. to 105° F. in less than half an hour, after which the temperature remained stationary (with slight fluctuations) until near death.

All the experiments point to the fact that abrus-globulin produces, when subcutaneously inoculated, a remarkable fall of temperature in pigeons; at the same time producing in them great rapidity of respiration, a phenomenon also observed in mammals.

Effect on Blood Pressure.—The effect of abrus-globulin on the blood-pressure was tested in dogs. Thus, in one experiment, where a dose of 0.01 gram per kilo. of body weight was injected into the femoral vein, there was no effect on the blood pressure in the carotid artery nor on the inhibitory power of the vagus, which was tested 15 minutes,

25 minutes, and 37 minutes after the inoculation. In only two cases have we noticed any effect; in both these there was a very slight and gradual fall in the blood pressure. In one of these cases a dose of 0·044 gram per kilo. of body weight was injected into the femoral vein, and in the other a dose of only 0·0042 gram per kilo.

There is, therefore, no marked effect on the blood pressure, nor on the inhibitory power of the vagus, such as we noticed on the body temperature. The only immediate evident effect of abrus-poisoning is a fall of the temperature; symptoms of weakness and of rapid respiration only appear much later, from five to twelve hours after the subcutaneous inoculation, according to the dose given.

Effect of Heat on the Physiological Action of Abrus-globulin.—Boiling destroys the activity of a watery extract of abrus seed. We have performed many experiments with the view of determining at exactly what temperature the activity of the poison is destroyed. It is reasonable to suppose that if the chief poisonous principle of abrus seed is the globulin, that the activity would be destroyed at the temperature at which the globulin coagulates; and this we have found to be the case.

The Poisonous Action of Abrus-globulin is completely destroyed by momentarily heating a Solution to a Temperature of 75° or 80° C., the Temperature at which the Globulin coagulates.

The heating was done in a test-tube placed in a water-bath; as soon as the solution of globulin was at the required temperature, the tube was removed and rapidly cooled.

In one experiment, four rats were inoculated with the same dose of globulin, which in one case had been heated to 50° C., in another to 60° C., in the third to 70° C., whilst the fourth was unheated; all the animals died with symptoms of abrus-poisoning within thirty hours of the inoculation. In another experiment, three rats were inoculated, the first with a solution of globulin heated to 75° C., the second with the same amount heated to 80° C., whilst the third was inoculated with unheated globulin. The first two rats, those inoculated with solutions heated to 75° and 80° C., remained quite well, while the third, inoculated with unheated globulin, died within twenty-four hours of abrus-poisoning.

Experiments performed on the eye with solutions of globulin, similarly treated, gave the same results; if heated up to 50°, 60°, or 70° C., the globulin produces as intense an ophthalmia as when unheated, but if heated to 75° or 80°, the globulin produces no inflammation or œdema.

The *conclusions*, therefore, at which we have arrived from our experiments are—

1. That the poisonous principle of the seeds of *Abrus precatorius* (jequirity) is a globulin.*

2. That the activity of this globulin is destroyed by heating its solution to 75° or 80° C., the temperature at which it coagulates.

3. That this globulin produces a remarkable fall of body temperature after subcutaneous injection.

4. That it causes rapidity of breathing shortly before death.

5. That the other actions ascribable to the globulin are: the production of local cedema and inflammation when subcutaneously injected or applied to the eye, the presence, *post-mortem*, of petechiæ beneath the serous membrane, and the occurrence of hæmorrhagic gastro-enteritis.

III. "The Toxic Action of the Albumose from the Seeds of *Abrus precatorius*." By SIDNEY MARTIN, M.D. Lond., British Medical Association Research Scholar, Assistant Physician to the Victoria Park Chest Hospital. (From the Physiological Laboratory, University College, London.) Communicated by E. A. SCHÄFER, F.R.S. Received May 8, 1889.

An account, by Dr. Wolfenden and myself, of the physiological action of the globulin which I extracted from the seeds of the jequirity plant, has been presented the Royal Society. I have shown† that there are two proteids present in the seeds; a globulin and an albumose. The present paper deals with the physiological action of the albumose.

Dr. Wolfenden and I showed in the paper referred to that the globulin possessed the poisonous qualities of the watery extract of the seeds and of the body called "abrin," described by Drs. Warden and Waddell. After being obtained in the pure state, it produced severe conjunctivitis when applied to the eye, and when subcutaneously injected it caused local cedema and ecchymosis, followed by death with the signs and symptoms of gastro-intestinal irritation and inflammation. It moreover lowered the body-temperature of the pigeon in a remarkable manner. From the method used by Drs. Warden and Waddell in preparing their "abrin," both proteids would be obtained, since they used a watery extract and precipitated the proteids with alcohol. Abrin would, therefore, be a mixture of globulin and albumose. As Dr. Wolfenden and I had found that the

* An account of the physiological action of the albumose of abrus-seeds has been presented to the Royal Society by one of us (M.).—May 10, 1889.

† 'Roy. Soc. Proc.,' vol. 42, p. 331.

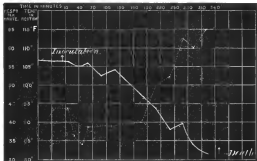


FIG. 1.—Pigeon weighing 216 grams: 0.2 gram streptococci injected over pectoral muscle (0.025 gram per kilo. of body weight). Death in 5 hours 45 mins. Temperature curve, thick line; respiration curve, dotted line.